Health Canada Santé Canada

Your health and safety... our priority. *Votre santé et votre sécurité... notre priorité.*

Proposed Registration Decision PRD2011-18

Pseudomonas fluorescens Strain A506

(publié aussi en français)

23 September 2011

This document is published by the Health Canada Pest Management Regulatory Agency. For further information, please contact:

Publications
Pest Management Regulatory Agency
Health Canada
2720 Riverside Drive
A.L. 6604-E2
Ottawa, Ontario
K1A 0K9

Internet: pmra.publications@hc-sc.gc.ca
healthcanada.gc.ca/pmra
Facsimile: 613-736-3758
Information Service:
1-800-267-6315 or 613-736-3799
pmra.infoserv@hc-sc.gc.ca

Canada

ISSN: 1925-0878 (print)
1925-0886 (online)

Catalogue number: H113-9/2011-18E (print version)
H113-9/2011-18E-PDF (PDF version)

© Her Majesty the Queen in Right of Canada, represented by the Minister of Health Canada, 2011

All rights reserved. No part of this information (publication or product) may be reproduced or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, or stored in a retrieval system, without prior written permission of the Minister of Public Works and Government Services Canada, Ottawa, Ontario K1A 0S5.

Table of Contents

Overview 1
Proposed Registration Decision for *Pseudomonas fluorescens* Strain A506 1
What Does Health Canada Consider When Making a Registration Decision? 1
What is *Pseudomonas fluorescens* strain A506? 2
Health Considerations 2
Environmental Considerations 4
Value Considerations 4
Measures to Minimize Risk 4
Next Steps 5
Other Information 5
Science Evaluation 7
1.0 The Active Ingredient, Its Properties and Uses 7
1.1 Identity of the Active Ingredient 7
1.2 Physical and Chemical Properties of the Active Ingredients and End-use Product 8
2.0 Methods of Analysis 8
2.1 Methods for Analysis of the Active Ingredient 8
2.2 Methods for Establishment of Purity of Seed Stock 9
2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products 9
2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites 9
2.5 Methods for Determination of Relevant Impurities in the Manufactured Material 9
2.6 Methods to Show Absence of Any Human and Mammalian Pathogens 10
2.7 Methods to Determine Storage Stability, Shelf-life of the Microorganism 10
3.0 Impact on Human and Animal Health 10
3.1 Toxicity and Infectivity Summary 10
3.2 Occupational/Bystander Exposure and Risk Assessment 15
3.2.1 Occupational 15
3.2.2 Bystander 15
3.3 Dietary Exposure and Risk Assessment 16
3.3.1 Food 16
3.3.2 Drinking Water 16
3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations 16
3.4 Maximum Residue Limits 17
3.5 Aggregate Exposure 17
3.6 Cumulative Effects 18
4.0 Impact on the Environment 18
5.0 Value 18
6.0 Pest Control Product policy Considerations 18
6.1 Toxic Substances Management Policy Considerations 18
6.2 Formulants of Health Concern 19

7.0 Summary 20
7.1 Methods for Analysis of the Microorganism as Manufactured 20
7.2 Human Health and Safety 20
8.0 Proposed Regulatory Decision 22
List of Abbreviations 23
Appendix I Tables and Figures 25
1.0 Toxicity and Infectivity of *Pseudomonas fluorescens* strain A506 and its associated end-use product Blightban A506 25
References 27

Overview

Proposed Registration Decision for *Pseudomonas fluorescens* Strain A506

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Pseudomonas fluorescens* strain A506 and Blightban A506, containing the technical grade active ingredient *Pseudomonas fluorescens* strain A506, to control fire blight on apples and pears.

Pseudomonas fluorescens Strain A506 (Registration Number 29284) and Blightban A506 (Registration Number 29285) are conditionally registered in Canada. The detailed review for *Pseudomonas fluorescens* strain A506 and Blightban A506 can be found in Evaluation Report ERC2010-07, *Pseudomonas fluorescens* strain A506. Subsequent to the original applications, an application to register *Pseudomonas fluorescens* strain A506 (Registration Number 29284) was reviewed and conditionally approved. The current applications were submitted to convert *Pseudomonas fluorescens* strain A506, and Blightban A506 from conditional registration to full registration.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation section provides detailed technical information on the human health, environmental and value assessments of *Pseudomonas fluorescens* strain A506 and Blightban A506.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

---

1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact."

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

Before making a final registration decision on *Pseudomonas fluorescens* strain A506, the PMRA will consider all comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Pseudomonas fluorescens* strain A506, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What is *Pseudomonas fluorescens* strain A506?

Pseudomonas fluorescens strain A506 is a naturally occurring strain of bacterium that is used as a microbial pest control agent (MPCA) for the suppression of *Erwinia amylovora*, the pathogen that causes fireblight on apple and pear trees. Blightban A506 is a commercial end-use product containing *Pseudomonas fluorescens* strain A506 as the active ingredient. *Pseudomonas fluorescens* strain A506 works by competitively excluding the pathogen for space and nutrients on branches and leaves and on blossoms.

Health Considerations

Can Approved Uses of *Pseudomonas fluorescens* strain A506 Affect Human Health?

***Pseudomonas fluorescens* strain A506 is unlikely to affect your health when Blightban A506 is used according to the label directions.**

People could be exposed to *Pseudomonas fluorescens* strain A506 when handling and applying Blightban A506. The PMRA considers several key factors when assessing health risks: the microorganism's biological properties (for example, production of toxic by-products), reports of any adverse incidents, its potential for pathogenicity, infectivity and toxicity as determined in toxicological studies as well as the likely levels to which people may be exposed to this strain relative to exposures already encountered in nature to other strains of this microorganism.

---

3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Pseudomonas fluorescens strain AGS 3001.2, a strain similar to *Pseudomonas fluorescens* strain A506, was found to be mildly irritating to the skin; therefore the precautionary words "CAUTION: Skin irritant" are required on the technical and end-use product labels.

No other significant toxicity or signs of disease were observed in the toxicity studies submitted to support *Pseudomonas fluorescens* strain A506.

Residues in Water and Food

Dietary risks from food and water are not of concern

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

Strains of *Pseudomonas fluorescens* are common in nature. While the use of Blightban A506 in orchards will lead to transient increases in populations, over the long-term, it is not expected to significantly increase natural environmental background levels of this microorganism. Furthermore, no significant adverse effects were reported when a mixture containing *Pseudomonas fluorescens* strain A506 and other *Pseudomonas* strains, or when *Pseudomonas fluorescens* strain AGS 3001.2, a strain similar to *Pseudomonas fluorescens* strain A506, was administered orally to rats.

Also, given that Blightban A506 is applied to pome fruit trees at the flowering stage, *Pseudomonas fluorescens* strain A506 is not expected to come into direct contact with fruit, and consequently, the proposed food use pattern is unlikely to result in significant residues on fruit at the time of harvest. Consequently, dietary exposure is minimal to non-existent, and the establishment of an MRL is not therefore required for *Pseudomonas fluorescens* strain A506. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

Occupational Risks From Handling Blightban A506

Occupational risks are not of concern when Blightban A506 is used according to label directions, which include protective measures

Workers using Blightban A506 can come into direct contact with *Pseudomonas fluorescens* strain A506 on the skin, in the eyes, or by inhalation. For this reason, the label will specify that users exposed to Blightban A506 must wear waterproof gloves, long-sleeved shirts, long pants, a NIOSH approved respirator (with any N–95, R–95, P–95 or HE filter), and shoes plus socks. Early-entry workers will also be restricted from entering areas where Blightban A506 has been applied for a period of 4 hours unless wearing the indicated personal protective equipment.

For bystanders, exposure is expected to be much less than that of workers involved in loading and application activities and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When Blightban A506 Is Introduced Into the Environment?

Environmental risks are not of concern.

Based on the limited use pattern of Blightban A506 in apple and pear orchards, natural populations of *Pseudomonas fluorescens* strain A506 are not expected to considerably increase in the terrestrial environment, and indirect exposure to aquatic environments is expected to be minimal. Cases of infection or disease in animals associated with other strains of *Pseudomonas fluorescens* are few, and none involved strain A506. Also, *Pseudomonas fluorescens* strain A506 is not expected to be pathogenic to plants.

Value Considerations

What Is the Value of Blightban A506?

Blightban A506 is a new biological pesticide that may be used with Streptomycin 17, a bactericide currently registered for fire blight control which has important resistance management issues. Blightban A506 is compatible with streptomycin and should be used in an integrated fire blight suppression program. Suppression of the fire blight pathogen, *Erwinia amylovora*, with Blightban A506 will help reduce grower reliance on streptomycin.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

Key risk-reduction measures previously recommended by the PMRA for the label of Blightban A506 are listed in ERC2010-07.

Key Risk-Reduction Measures

Human Health

Because of concerns with users developing allergic reactions through repeated high exposures to *Pseudomonas fluorescens* strain A506, anyone handling, mixing/loading, or involved in clean-up/repair activities of Blightban A506 must wear waterproof gloves, a long-sleeved shirt, long pants and a dust/mist filtering respirator/mask (MSH/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any N-95, R-95, P-95 or HE filter.

Environment

Environmental risk mitigation measure can be found under Evaluation Report ERC2010-07 *Pseudomonas fluorescens* strain A506.

Next Steps

Before making a final registration decision on *Pseudomonas fluorescens* strain A506, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Pseudomonas fluorescens Strain A506* (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Pseudomonas fluorescens strain A506

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Pseudomonas fluorescens* strain A506
Function	Suppress the fireblight pathogen, *Erwinia amylovora,* in commercial apple and pear orchards
Binomial name	*Pseudomonas fluorescens* strain A506
Taxonomic designation	
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	*Pseudomonas*
Species	*fluorescens*
Strain	A506
Patent Status information	Canadian Patent No. CA1192154
Minimum purity of active	5.0×10^{10} CFU/g
Identity of relevant impurities of toxicological, environmental and/or significance.	The technical grade active ingredient does not contain any impurities or micro contaminants known to be TSMP Track 1 substances.

1.2 Physical and Chemical Properties of the Active Ingredients and End-use Product

As Blightban A506 is manufactured following a continuous process, there is no true intermediate stand-alone technical grade active ingredient.

End-use Product—Blightban A506

Property	Result
Colour	White
Odour	odourless
Physical state	Fine powder, freeze-dried
Formulation type	Live organism
Guarantee	1×10^{10} CFU/g
pH	6.7 (5% slurry)
Density	0.4 g/cm^3
Storage stability	1 week at 21°C, or 1 month at 4°C, or up to 9 months at -27°C
Corrosion characteristics	Non-corrosive
Explodability	Non-explosive

2.0 Methods of Analysis

2.1 Methods for Analysis of the Active Ingredient

Pseudomonas fluorescens strain A506 is identified to the species level, in Biotype A, by biochemical characteristics, colony morphology, and molecular methods which use polymerase chain reactions (PCR) coupled with multiple enzyme restriction fragment length polymorphism (PCR-MERFLP) analysis and phylogenetic analyses of nucleotide sequences of *Pseudomonas*-specific genes.

Strain-specific identification of the MPCA is accomplished using a series of classical microbial identification strategies which include phenotypic tests and molecular identification methods to positively identify an isolate as *P. fluorescens* strain A506. The criteria include colony characteristics (colour, morphology, pigment production); an antibiotic resistance assay; production of antibiotics, proteins and lipopeptides in culture; biochemical tests (tryptophane side chain oxidase, indole acetic acid); and genomic comparisons of the *strB* resistance gene, as well as of a universal regulatory gene (*rpoS*) for a strain-specific point mutation.

2.2 Methods for Establishment of Purity of Seed Stock

A stock of *Pseudomonas fluorescens* strain A506 is maintained frozen in liquid nitrogen and is used to generate a primary mother culture. From the primary mother culture, a secondary mother culture is sub-cultured and used for inoculation of the fermentation mixture. A culture is also stored at the American Type Culture Collection (ATCC) as accession number ATCC 31948.

Practices for ensuring the purity of the seed stock were adequately described in the method of manufacture and quality assurance program.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The potency (CFU/g) of *Pseudomonas fluorescens* strain A506 is determined by plate counts on standard media with antibiotics with which the MPCA is resistant.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

Pseudomonas fluorescens is ubiquitous in nature, and has been isolated from a wide variety of environments. The use of Blightban A506 (containing *Pseudomonas fluorescens* strain A506) in orchards is not expected to significantly increase the natural environmental background levels of this microorganism. No adverse effects from dietary exposure have been attributed to natural populations of *Pseudomonas fluorescens* and none were observed during acute oral toxicity testing neither with a formulation containing *Pseudomonas fluorescens* strain A506 nor with a strain equivalent to the MPCA. There are no reports of mammalian toxins being produced by the MPCA. Furthermore, as Blightban A506 is applied to pome fruit trees at the flowering stage, *Pseudomonas fluorescens* strain A506 is not expected to come into direct contact with fruit, and consequently, the proposed food use pattern is unlikely to result in significant residues on fruit at the time of harvest. The establishment of a maximum residue limit (MRL) is therefore not required for *Pseudomonas fluorescens* strain A506.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during manufacture of Blightban A506 are acceptable.

Contamination is monitored throughout manufacturing using total plate counts on standard media following standard methods (incubation at 25°C). Gross contamination would also be distinguished by microscopic examination of the product.

The absence of human pathogens, and below-threshold levels of contaminants is ensured throughout manufacturing using pathogen specific media. Release standards for microbial contaminants in the end-use product comply with those permitted by the Agency and are adequate for detecting human and animal microorganisms of concern.

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted previously, microbe-specific screening methods for enteric bacteria/total coliforms, yeasts/moulds, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Vibrio* spp. and *Pseudomonas aeruginosa* are adequate for detecting and enumerating microbial contaminants of concern and to ensure that Blightban A506 does not contain unacceptable levels of human and animal disease-causing microorganisms.

2.7 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Results from storage stability testing from four lots of Blightban A506 showed that the end-use product is stable for one week at 21°C. Alternatively, the end-use product is stable for 1 month at 4°C, or for up to 9 months at -27°C.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

A survey of published literature identified several clinical cases of transfusion-related septicæmia caused by *Pseudomonas fluorescens* from contaminated blood products or intravenous fluids, or improper vene-puncture technique. Many of the cases occurred in patients who were severely immuno-compromised or cancer patients and had fatal outcomes. Certain clinical cases of *Pseudomonas fluorescens* bacteræmia were also linked to contaminated medical supplies, or the formation of biofilms in catheters or similar equipment. Other reports included a fatal case of neonatal sepsis caused by *Pseudomonas fluorescens* with no apparent conditions to predispose the infant to infection, and a case of neonatal non-hematogenous osteomyelitis of the sternum following a sternotomy. There was also a case of osteolytic lesions in the upper extremities of an elderly cancer patient. Another case reported on *Pseudomonas fluorescens*-associated endophthalmitis in an eye that was punctured with a rusty metal spring. *Pseudomonas fluorescens* has also been identified as an emerging bacterial pathogen in patients with human immunodeficiency virus, and may play a role in pathogenesis of inflammatory bowel diseases.

While there have been some reports of clinical cases associated with *Pseudomonas fluorescens* infection, most of the cases were transfusion-related arising from contaminated intravenous products in compromised patients. *Pseudomonas fluorescens* strain A506 in Blightban A506 was originally obtained from healthy plant tissue, and while it cannot be stated without qualification that the exact strain in Blightban A506 occurs worldwide, it is likely that functionally equivalent strains of *Pseudomonas fluorescens* are distributed worldwide. Consequently, humans and other animals are considered to be continuously exposed to natural populations of *Pseudomonas fluorescens* by dermal exposure, and by ingestion of leafy plant materials, or fruits and vegetables or other edible plant organs. Given the prevalence of *Pseudomonas fluorescens* in the environment, the number of clinical cases associated with *Pseudomonas fluorescens* are considered very low.

In general, *Pseudomonas fluorescens* is regarded as one of the least virulent members of Pseudomonaceae family, while *Pseudomonas aeruginosa* is regarded as the most common, and most virulent, pathogen of the family. The limited growth of *Pseudomonas fluorescens* strain A506 at temperatures > 37°C further reduces its potential as an opportunistic pathogen in humans.

Other reports pertained to the inflammatory potential of *Pseudomonas fluorescens*. As a gram-negative bacterium, lipopolysaccharide (LPS) in the cell wall of *Pseudomonas fluorescens* can elicit immune responses in response to the endotoxin. However, the inflammatory potential of LPS of *Pseudomonas fluorescens* was shown to be moderately reactive compared to *Streptococcus fecalis* and *Clostridium perfringens*, for example, which showed higher inflammatory reactions. Exposure to organic dusts, containing *Pseudomonas fluorescens* and other gram-negative bacteria, have led to respiratory disease, such as asthma, allergy, hypersensitivity pneumonitis and toxic pneumonitis (organic dust toxic syndrome).

The PMRA conducted a detailed review of the toxicological database submitted in support of *Pseudomonas fluorescens* strain A506. The database is largely complete, consisting of laboratory animal (*in vivo*) toxicity studies (acute oral toxicity, acute inhalation toxicity and dermal irritation; and acute oral toxicity, intraperitoneal injection) currently required for health hazard assessment purposes. These studies were carried out in accordance with currently accepted international testing protocols and good laboratory practices. Waiver requests were deemed acceptable to address dermal toxicity, as well as to address the infectivity potential of the MPCA, in lieu of testing. In addition to the required studies, an eye irritation study using an isolate equivalent to the MPCA was also submitted. The scientific quality of the database is considered sufficient to characterize the toxicity of this pest control agent and end-use product.

The studies submitted for human health and safety testing for Blightban A506 were originally generated for the USEPA's registration of a series of other products, namely Frostban A, Frostban B, Frostban C and Frostban D. The Frostban A formulation is such that it contains a mixture of three *Pseudomonas* spp. strains, namely *Pseudomonas fluorescens* strain A506 (Frostban B; equivalent to the Blightban A506 TGAI), *Pseudomonas syringae* strain 742RS (Frostban C), and *Pseudomonas fluorescens* strain 1629RS (Frostban D) plus formulants. Consequently, the acute oral toxicity study and the intraperitoneal infectivity study submitted for Blightban A506 were conducted with Frostban A, rather than with the proposed MCPA. However, the data were considered acceptable to support the toxicity requirements for Blightban A506 since Frostban A contains the MPCA in BlightBan A506, in addition to other species of *Pseudomonas* which are known to be more pathogenic than the MPCA (for example, *Pseudomonas syringae*), and since the manufacturing process is such that there is no true MCPA for this end-use product. Furthermore, based on the level of dosing and the relative proportion of *Pseudomonas fluorescens* strain A506 in Frostban A, the administered doses were above the recommended doses for oral (10^8 CFU/animal) and intraperitoneal injection (10^7 CFU/animal) testing with a MPCA.

The test substance in the acute oral toxicity study, the acute inhalation toxicity, dermal irritation and eye irritation studies was *Pseudomonas fluorescens* strain AGS 3001.2 (also referred to as *Pseudomonas fluorescens strain* GJP17BR2). Since *Pseudomonas fluorescens* strain AGS3001.2 does not have any taxonomical differences to *Pseudomonas fluorescens* strain 1629RS, and since data from the Part 2 Product Characterization and Analysis database demonstrates that strain 1629RS and the MPCA are equivalent in their metabolic characteristics, strain 1629RS and strain A506 can be considered very closely related. Furthermore, AGS3001.2 and Frostban A (containing strain A506) both showed low toxicity via the oral route, which demonstrates that AGS3001.2 and *Pseudomonas fluorescens* strain A506 can be considered toxicologically equivalent. Also, clinical cases of infection from *Pseudomonas fluorescens* in published literature have been opportunistic in nature, which suggests that *Pseudomonas fluorescens* is not a particularly invasive or virulent pathogen and further suggests lack of infectivity of the MPCA. Toxicity testing with AGS3001.2 is expected to provide relevant information on the toxicological nature of *Pseudomonas fluorescens* strain A506 and consequently, toxicity testing via the oral and inhalation routes of exposure, and dermal and eye irritation with *Pseudomonas fluorescens* strain AGS3001.2 is considered acceptable. The USEPA also accepted testing with *Pseudomonas fluorescens* strain AGS3001.2 in support of Frostban B (EPA Reg. No. 64004-2; PMRA 1393493).

In an acute oral toxicity study (limit test), fasted, Sprague-Dawley rats were given a single oral dose of Frostban A at 8.4×10^{10} CFU/animal (measured) and observed for 7 days. No mortalities occurred, and there were no signs of toxicity and no abnormalities upon gross necropsy. Frostban A was considered to be of low toxicity in the rat by the oral route. As no interim sacrifices for microbial enumeration of tissues/organs/body fluids were performed, infectivity of the MPCA was not assessed.

An acute oral toxicity study with *Pseudomonas fluorescens* strain AGS3001.2 was also conducted. Fasted, young adult Sprague-Dawley rats were given a single oral dose of undiluted AGS3001.2 at 5.0 g/kg bw and observed for 18 days. No mortalities occurred, there were no treatment related clinical signs, or necropsy findings, and all animals gained weight throughout the study. *Pseudomonas fluorescens* strain AGS3001.2 was considered to be of low toxicity to the rat by the oral route of exposure. Infectivity however, was not assessed directly.

In a pulmonary toxicity study, young, Sprague-Dawley rats were exposed to undiluted AGS3001.2 at 5.3 mg/L (measured) for 4 hours by whole body exposure and observed for 14 days. No mortalities occurred. There were no unusual signs of toxicity reported other than expected signs of discomfort following inhalation exposure (all animals on Day 1 only) and slight corneal opacity in one animal (Day 2 only). *Pseudomonas fluorescens* strain AGS3001.2 was considered to be of low toxicity to the rat by the inhalation route of exposure. Infectivity however, was not assessed directly.

In an intraperitoneal injection study, Swiss Webster mice were injected with Frostban A at 2.1×10^8 CFU/animal (measured) and observed for 7 days. There were no mortalities. General toxicity after dosing was observed in all animals after dosing but had cleared in all but one female by Day 7. As no interim sacrifices for microbial enumeration of tissues/organs/body fluids were performed, infectivity of the MPCA was not assessed. Although guidelines recommend that bacterial MPCAs be tested by intravenous (I.V.) injection, testing via intraperitoneal injection was considered appropriate for Frostban A since the immune reaction to the lipopolysaccharide (LPS) of gram-negative bacteria (such as *Pseudomonas fluorescens*) following an I.V. injection could mask any potential symptoms resulting from an actual infection by the MPCA.

A waiver request from an infectivity study using either the pulmonary instillation, or intravenous, route of administration was deemed acceptable based on the results from toxicity testing via the oral, inhalation and intraperitoneal routes, and based on the fact that the MPCA does not survive at the human body temperature, and that the clinical cases of true infection associated with *Pseudomonas fluorescens* were mostly transfusion-related incidents arising from contaminated intravenous products in immunocompromised patients only. Also, literature indicated that there have been no cases of infection in root-crop processing workers exposed to *P. fluorescens* at much higher concentrations than those expected during application of Blightban A506. Since *Pseudomonas fluorescens* strain A506 is a naturally occurring strain in the environment, it is expected that humans and other animals are exposed to natural populations of *Pseudomonas fluorescens*. In general, *Pseudomonas fluorescens* is regarded as one of the least virulent members of Pseudomonaceae family, and recent reclassification has placed the species into RNA homology group I which excludes any clinically significant Pseudomonads. Based on this information the waiver request was deemed acceptable to address the infectivity potential of *P. fluorescens* strain A506.

In a dermal irritation study (limit test), six young adult male New Zealand white rabbits were exposed to 0.5 mL of undiluted *Pseudomonas fluorescens* strain AGS3001.2 for 4 hours at two shaved skin sites and observed for 14 days. Irritation was scored by the method of Draize (1965). Moderate erythema with very slight edema was noted until Day 2 after which signs of irritation tapered off to very slight erythema by Day 10 or sooner. All signs of irritation were resolved by Day 14 or sooner. *Pseudomonas fluorescens* AGS3001.2 was considered to be mildly irritating to the skin based on the Maximum Irritation Score (MIS) of 1.67. The signal words "CAUTION: Skin Irritant" will be required on the technical label and on the end-use product label.

A request to waive dermal toxicity testing of Blightban A506 was based on the lack of toxicity of a substance equivalent to the MPCA via the oral, and inhalation routes of exposure, and a lack of toxicity with a formulation containing the MPCA (as well as other more pathogenic species such as *P. syringae*), via the oral and intraperitoneal injection route of exposure. In particular, the inhalation exposure was by full body exposure (>24 hours) which is considered representative of dermal toxicity testing.

The results from dermal irritation testing were also considered. Precautionary labelling as a result from dermal irritation testing will alert users of the potential for dermal irritation ("Caution: skin irritant") from the technical and end-use product, and the requirement for personal protective equipment during all handling of Blightban A506 will further reduce the likelihood of occupational dermal exposure. These label statements are expected to adequately reduce the potential for dermal toxicity from Blightban A506. Furthermore, none of the formulants in the end-use product pose a concern with respect to dermal toxicity. For these reasons, dermal irritation testing with a test substance equivalent to the MPCA is considered acceptable in lieu of dermal toxicity testing with the end-use product and the waiver request was deemed acceptable.

In an eye irritation study (limit test), 0.1 mL of undiluted *Pseudomonas fluorescens* strain AGS3001.2 was instilled into the conjunctival sac of one eye of six young adult male New Zealand white rabbits and observed for 72 hours. Irritation was scored by the method of Driaze (1959). All animals experienced some sign of irritation up to the 24-hour timepoint, but all signs of irritation cleared in all treated eyes by 72-hours or sooner. Based on the Mean Average Score (MAS) of 2.56 and the MIS of 10.67 (at 1 hour), AGS3001.2 was classified as non- to minimally irritating to the eyes of rabbits.

As previously mentioned, *Pseudomonas fluorescens* strain A506 is also a known sensitizing component of agricultural dusts due to the lipopolysaccharide (LPS) found in the cell envelope of all gram-negative bacteria. There were two incidents of hypersensitivity reported which arose in workers after they were exposed to dust generated during product manufacture. Since the time of the incidents, certain modifications have been made to the manufacturing process to minimize worker exposure to the dust.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA and because there were no obvious indications of infectivity toxicity or pathogenicity in the test animals treated in the Tier I acute oral, and intraperitoneal injection toxicity tests.

Within the available scientific literature, there are no reports that suggest *P. fluorescens* strain A506 has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral, intraperitoneal and pulmonary routes of exposure, the immune system is still intact and able to process the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated from exposure to *P. fluorescens* strain A506.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential routes of handler exposure to *Pseudomonas fluorescens* strain A506 are pulmonary, dermal and to some extent ocular, with the primary source of exposure to workers being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Pseudomonas fluorescens* is not generally identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. As a strain equivalent to *Pseudomonas fluorescens* strain A506 showed mild irritation by the dermal route, precautionary labelling will notify users of the potential for dermal irritation, and the label requirement for personal protective equipment will further reduce the potential for dermal exposure.

Eye irritation from *Pseudomonas fluorescens* strain A506 is not expected based on results from testing.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity (allergic) reactions, and that individuals exposed to large quantities of *Pseudomonas fluorescens* strain A506 could possibly develop respiratory hypersensitivity upon repeated exposure to the product. Label statements (for example, Potential Sensitizer) and risk mitigation measures are required to protect workers who are likely to be exposed to the products. Such exposure to workers can be minimized if they wear waterproof gloves, long-sleeved shirts, long pants, shoes and socks, as well as a dust/mist filtering respirator/mask.

3.2.2 Bystander

Inhalation or dermal exposure to the general public from the proposed use of Blightban A506 in commercial orchards is expected to the low. Overall the Agency does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity profile for *Pseudomonas fluorescens* strain A506 and the assumption that precautionary label statements will be followed in the use of Blightban A506 to minimize off-target spray drift.

The label does not allow applications to turf, residential or recreational areas; therefore, non-occupational dermal exposure and risks to adults, infants, and children are low. Because the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Negligible to no risk is expected for the general population, including infants and children or animals, from residues in or on agricultural commodities since the timing of application of Blightban A506 to pome fruit trees at the flowering stage makes it unlikely that residues will be present on fruit at the time of harvest. Furthermore, *Pseudomonas fluorescens* strain A506 demonstrated no oral toxicity at the maximum dose tested in the Tier I acute oral toxicity study. Also, *Pseudomonas fluorescens* species are ubiquitous in nature and have been isolated from a wide variety of environments (including from orchards), with no adverse effects attributed to natural populations of *Pseudomonas fluorescens* reported from dietary exposure. Consequently, the establishment of an MRL is not required for *Pseudomonas fluorescens* strain A506.

Furthermore, higher tiered subchronic and chronic dietary exposure studies are not required because of the low toxicity of the MPCA in the test animals treated in the Tier I acute oral and pulmonary toxicity studies. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

Although *Pseudomonas fluorescens* strain A506 could enter neighbouring aquatic environments via spray drift or surface-water runoff, no risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and there were no harmful effects observed in animals that were exposed orally in Tier I acute oral toxicity tests. The Blightban A506 label advises users to limit spray drift and surface-water runoff. The potential transfer of *Pseudomonas fluorescens* strain A506 to surface or groundwater during runoff is considered minimal to non-existent due in part to its percolation through, and resulting capture, in soil where *Pseudomonas fluorescens* is well-adapted for growth. The Blightban A506 label also instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes. Also, municipal treatment of drinking water will likely remove the transfer of residues to drinking water. Therefore, potential exposure to *Pseudomonas fluorescens* strain A506 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e., no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the Agency concludes that *Pseudomonas fluorescens* strain A506 is of low toxicity, does not appear to be pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the

general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, do not apply to this MPCA. As a result, the Agency has not used a margin of exposure (safety) approach to assess the risks of *Pseudomonas fluorescens* strain A506 to human health.

3.4 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a MRL under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

The commercial application of Blightban A506 to pome fruit trees at the flowering stage is unlikely to result in significant residues on treated fruit at the time of harvest. Furthermore, no adverse effects were observed in the acute oral toxicity study with *P. fluorescens* strain A506, and no adverse effects from dietary exposure have been attributed to natural populations of *P. fluorescens*. Therefore, the establishment of an MRL is not required for *P. fluorescens* strain A506.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information on *Pseudomonas fluorescens* strain A506, there is reasonable certainty that no harm will result from aggregate exposure of residues of *Pseudomonas fluorescens* strain A506 to the general Canadian population, including infants and children, when Blightban A506 is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Furthermore, few adverse effects from exposure to other isolates of *Pseudomonas fluorescens* encountered in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Blightban A506, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. There are no strains of *Pseudomonas fluorescens* or closely-related species currently registered for pest control purposes in Canada. Besides other naturally occurring strains of *Pseudomonas fluorescens* in the environment, the Agency is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity. Therefore, no cumulative effects are anticipated if the residues of *Pseudomonas fluorescens* strain A506 interact with related strains of this microbial species.

Incidents Reports

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the PMRA website. Incidents from Canada were searched and reviewed for *Pseudomonas fluorescens* strain A506.

As of February 8, 2011, there have been no incidents reported for products containing *Pseudomonas fluorescens* strain A506.

4.0 Impact on the Environment

An environmental review was not required for the conversion of BlightBan A506 from conditional to full registration. A full environmental summary can be found in Evaluation Report ERC2010-07 *Pseudomonas fluorescens* strain A506.

5.0 Value

A value review was not required for the conversion of BlightBan A506 from conditional to full registration. A full value summary can be found in Evaluation Report ERC2010-07 *Pseudomonas fluorescens* strain A506.

6.0 Pest Control Product policy Considerations

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's *Toxic Substances Management Policy* (TSMP), which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

In its review of *Pseudomonas fluorescens* strain A506, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy. Substances associated with its use were also considered, including microcontaminants in the technical product, *Pseudomonas fluorescens* strain A506 Technical, and formulants in the end-use product Blightban A506. The PMRA has reached the following conclusions:

Pseudomonas fluorescens strain A506 Technical does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track-1 criteria.

Therefore, the use of *Pseudomonas fluorescens* strain A506 Technical is not expected to result in the entry of Track 1 substances into the environment.

6.2 Formulants of Health Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*. The list is used as described in the PMRA Notice of Intent NOI2005-01 and is based on existing policies and regulations including: DIR99-03; Dir 2006-02, and taking into consideration the Ozone depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

The technical grade active ingredient (TGAI), *Pseudomonas fluorescens* strain A506 Technical, contains soy, which is identified in *Canada Gazette* Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health and Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions*. Therefore, the label for the technical product, *Pseudomonas fluorescens* strain A506 Technical, includes the precautionary statement "Warning: contains the allergen soy" on the principal display panel.

In addition to soy in the technical product, the end- use product Blightban A506 contains lactose (milk) which is identified in *Canada Gazette* Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health and Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions.* Therefore, the label for the end-use product, Blightban A506, includes the precautionary statement "Warning: contains the allergens soy and lactose (milk)" on the principal display panel.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulants initiatives and Regulatory Directive DIR2006-02.

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for *Pseudomonas fluorescens* strain A506 Technical and Blightban A506 are adequate to assess their safety to human health and the environment. The technical material was fully characterized and the specifications were supported by the analysis of a sufficient number of batches.

Strain-specific identification of the MPCA is accomplished using a series of classical microbial identification strategies which include phenotypic tests (for example, morphology, pigment production, antibiotic resistance, extracellular protein production, biochemical tests) and molecular identification methods (for example, gene analysis of the resistance gene *strB* and the regulatory gene *rpoS*) to positively identify an isolate as *P. fluorescens* strain A506.

Storage stability data were sufficient to support an expiration date of one week at 21°C, or 1 month at 4°C, or up to 9 months at -27°C.

7.2 Human Health and Safety

A formulation containing the MPCA, Frostban A, was of low toxicity in the rat by the oral route and there were no mortalities in mice injected intraperitoneally with Frostban A. However, infectivity was not assessed by either route.

A strain toxicologically equivalent to *Pseudomonas fluorescens* strain A506, *Pseudomonas fluorescens* strain AGS3001.2, was of low toxicity to the rat by the oral and pulmonary route of exposure and was non- to minimally irritating to the eyes of rabbits. As *Pseudomonas fluorescens* AGS3001.2 mildly irritating to the skin of rabbits, the signal words "CAUTION: Skin Irritant" will be required on the technical label and on the end-use product label.

The request to waive infectivity testing via the pulmonary instillation, or intravenous, route of administration was accepted based on the results from toxicity testing, the fact that the MPCA does not survive at the human body temperature and evidence from published literature which indicates that *P. fluorescens* has a low potential for pathogenicity in healthy individuals.

The request to waive dermal toxicity testing of Blightban A506 was accepted based on the lack of toxicity of *Pseudomonas fluorescens* strain AGS3001.2 via the oral route of exposure and the inhalation routes of exposure. Furthermore, Frostban A demonstrated a lack of toxicity via the oral and intraperitoneal injection route of exposure. Also, the label statements imposed as a result of dermal irritation testing and the standard label statements for personal protective equipment are expected to adequately reduce the potential for dermal toxicity from Blightban A506. Moreover, none of the formulants in the end-use product pose a concern with respect to dermal toxicity.

The technical product contains soy, and the end-use product contains lactose (milk), both of which are allergens known to cause anaphylactic type reactions. Therefore, the label for the technical and end-use product will include the precautionary statements "Warning: contains the allergen soy"and "Warning: contains the allergens soy and lactose (milk)" on the principal display panel, respectively.

When handled according to the label instructions, the potential routes of handler exposure are pulmonary, dermal and to some extent ocular, with the primary source of exposure to workers being dermal. Adverse effects from the inhalation and ocular routes of exposure are not expected based on results from testing. As a strain equivalent to *Pseudomonas fluorescens* strain A506 showed mild irritation by the dermal route, precautionary labelling will identify the technical and end-use product as a skin irritant. Furthermore, to reduce the potential for dermal exposure, the label will specify that users wear appropriate personal protective equipment and early-entry workers are restricted from entering areas where Blightban A506 has been applied for a period of 4 hours unless wearing the indicated personal protective equipment.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, and that exposure to allergens, including *Pseudomonas fluorescens* strain A506 may cause allergies following repeated exposures. As a result, the signal words "Potential Sensitizer" are required on the principal display panels of all technical and end-use products. Furthermore, personal protective equipment, including a NIOSH-approved respirator, is required to protect users that are likely to be primarily exposed to the products.

Because the use sites are limited to agricultural settings, bystander exposure is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

Negligible to no risk is expected for the general population, including infants and children or animals, from residues in or on agricultural commodities since the timing of application of Blightban A506 to pome fruit trees at the flowering stage makes it unlikely that residues will be present on fruit at the time of harvest. Furthermore, *Pseudomonas fluorescens* strain A506 demonstrated low toxicity at the maximum dose tested in the Tier I acute oral toxicity study, and no adverse effects have been attributed from dietary exposure to natural populations of *Pseudomonas fluorescens*. Consequently, the establishment of an MRL is not required for *Pseudomonas fluorescens* strain A506.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Pseudomonas fluorescens* strain A506 and Blightban A506 containing the technical grade active ingredient *Pseudomonas fluorescens* strain A506, to control fireblight on apples and pears. An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

°C	degree(s) Celcius
ADI	acceptable daily intake
ARD	acute reference dose
ATCC	American Type Culture Collection
bw	body weight
cm^3	centimetre cubed
CFU	colony forming units
DACO	data code
g	gram
g/kg bw	grams per kilogram body weight
kg	kilograms
LD_{50}	lethal dose 50%
LPS	lipopolysaccharide
MAS	maximum average score
MERFLP	multiple enzyme restriction fragment length polymorphism
MIS	maximum irritation score
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute of Occupational Safety and Health
PFGE	pulsed field gel electrophoresis
PCR	polymerase chain reaction
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	part per million
RFLP	restriction fragment length polymorphism
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy

Appendix I Tables and Figures

1.0 Toxicity and Infectivity of *Pseudomonas fluorescens* strain A506 and its associated end-use product Blightban A506

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Pseudomonas fluorescens* strain A506[1]				
Acute Oral Toxicity –Limit test	Rat –Sprague-Dawley 5/sex undiluted *Pseudomonas fluorescens* strain AGS3001.2 at 5.0 g/kg bw No control groups Observed for 18 days	Acute oral LD_{50} > 5.0 mg/kg bw (males, females)	-potency not reported -no mortalities no significant toxicity, no treatment related clinical signs, no necropsy findings or changes in bw LOW TOXICITY ACCEPTABLE	PMRA 1393488
Acute Pulmonary Toxicity- Limit Test	Rat –Sprague-Dawley 5/sex undiluted *Pseudomonas fluorescens* strain AGS3001.2 at 5.3 g/kg bw for 4 hours (whole body exposure) No control groups Observed for 14 days	Acute pulmonary LD_{50} >5.3 mg/L (males, females)	-potency not reported -no mortalities -general signs of discomfort in all animals on Day 1 only -slight corneal opacity in one animal on Day 2 only LOW TOXICITY ACCEPTABLE	PMRA 1393490
Acute Dermal Toxicity- Waiver	Waiver submitted. The waiver request was based on the lack of toxicity of a strain equivalent to the MPCA via the oral route, and via the inhalation routes of exposure which was a full body exposure for >24 hours; and on a lack of toxicity of a formulation containing the MPCA (as well as other more pathogenic species, such as *P. syringe*) via the oral and intraperitoneal injection routes of exposure. Furthermore, the label statements imposed from dermal irritation testing and standard label statement for personal protective equipment will to adequately reduce the potential for dermal toxicity from Blightban A506, and none of the formulants in the end-use product pose a concern with respect to dermal toxicity. WAIVER ACCEPTED			PMRA 1393487
Eye Irritation- Limit Test	Rabbit- New Zealand White (6 male animals) 0.1 mL undiluted *Pseudomonas fluorescens* strain AGS3001.2.into one eye The other eye served as the untreated control Eyes were not washed out Observed for 72 h	MIS (1 h)= 10.67 MAS= 2.56	-potency not reported -in all animals: some sign of irritation (opacity; swelling of the iris, slight erythema/swelling, discharge in the conjunctivae) up to 24 hrs -all signs cleared by 72 hrs or sooner NON- TO MINIMALLY	PMRA 1393495

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
			IRRITATING ACCEPTABLE	
Acute Toxicity/Irritation of Blightban A506[2]				
Acute Oral Toxicity –Limit Test	Rat –Sprague-Dawley 10/sex of Frostban A in phosphate buffer at 8.4×10^{10} CFU/animal (measured) Vehicle control group Observed for 7 days	acute LD_{50} $> 8.4 \times 10^{10}$ CFU/animal (males, females)	-no mortalities no significant toxicity, no signs of toxicity and no abnormalities upon gross necropsy -did not meet the recommended observation period of 21 days LOW TOXICITY ACCEPTABLE BUT SUPPLEMENTAL	PMRA 1393489
Acute Infectivity– Intraperitoneal Injection	Mouse –Swiss Webster 5/sex Frostban A in phosphate buffer at 2.0×10^{8} CFU/animal (measured) Vehicle control group Observed for 7 days	n/a, as infectivity was not assessed	-no mortalities -in all animals: general signs of toxicity (scruffy coats, discharge from eyes, lethargy, diarrhea) immediately after dosing -all signs had cleared by Day 7, except in one female animal (minor scruffy coat) -tested by I.P injection rather than by I.V. was acceptable given that an I.V. injection of the MPCA may cause an immune reaction (due to the LPS) which could mask symptoms of infection -did not meet the recommended observation period of 21 days -no interim sacrifices; infectivity was not assessed ACCEPTABLE BUT SUPPLEMENTAL	PMRA 1393491

bw: body weight
h: hour(s)
n/a: not applicable
MAS: Maximum Average Score
MIS: Maximum Irritation Score at a given time point
I.P.: intraperitoneal
I.V.: intravenous

[1] Testing was conducted with *Pseudomonas fluorescens* strain AGS3001.2, a strain considered equivalent to the MPCA (*Pseudomonas fluorescens* strain A506) rather than with the MPCA itself

[2] Testing was conducted with Frostbans A, a mixture containing the MPCA, as well as other *Pseudomonas* species which are known to be more pathogenic than the MPCA (for example, *P. syringae*), rather than with Blightban A506 itself

References

A. List of Studies/Information Submitted by the Registrant

1.0 METHODS OF ANALYSIS

PMRA 1948042 DACO: M2.7.1 [CBI document deleted]

2.0 IMPACT ON HUMAN AND ANIMAL HEALTH

PMRA 1948043 2010, Request for a Waiver from the Requirement of Providing a Toxicity and Infectivity Study on *Pseudomonas fluorescens* strain A506, DACO: M4.2

PMRA 1948044 Andersen, JL, 2008, *Pseudomonas fluorescens* Proposed Registration Review Decision Case 6006, DACO: M12.5

B. Additional Information Considered

i. Published Information

1.0 METHODS OF ANALYSIS

PMRA 2010818 2010, http://www.ncbi.nlm.nih.gov/nuccore/222430242, *Pseudomonas fluorescens* strain A506 truncated RpoS (rpoS) gene, complete cds. GenBank: FJ384964.1 Nucleotide Alphabet of Life., DACO: M2.7.1

2.0 HUMAN AND ANIMAL HEALTH

PMRA 1393405 Selvaraju, S.B. et al, 2005, Biocidal activity of formaldehyde and nonformaldehyde biocides toward Mycobacterium immunogenum and *Pseudomonas fluorescens* in pure and mixed suspensions in synthetic metalworking fluid and saline.

PMRA 1393416 Hacker, et al, 2005, Determination of the Inflammatory Potential of Bioaerosols by Using Human Whole Blood Cytokine Response.

PMRA 1393428 Bernstein, D.I. et al, 1995, Machine operator's lung. A hypersensitivity pneumonitis disorder associated with exposure to metalworking fluid aerosols. DACO: M2.7.2,M4.0,M8.0,M9.0

PMRA 1393430 Sigsgaard, T. et al, 2005, Microbial cell wall agents as an occupational hazard. Toxicology and Applied Pharmacology 207:

PMRA 1393454 Fishwick, David, et al, 2005, Respiratory symptoms, immunology and organism identification in contaminated metalworking fluid workers. What you see is not what you get.

PMRA 1659565 Skorska C et al, 2005, Exposure to Airborne Microorganisms, Dust and Endotoxin During Processing of Valerian Roots on Farms, DACO: M2.0,M4.0,M8.0,M9.0